EFFECTS OF FILTRATION ON THE POTENCIES OF ANTITOXINS

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The object of the work was to ascertain whether any antitoxic units were adsorbed when a product, such as tetanus antitoxin, was filtered through a Berkefeld type filter. Mechanical losses were not considered.

The need of comparing the filtered with original products was pointed out some time ago, by the Chief of the Bureau of Animal Industry, Dr. John R. Mohler.

Materials.—Table 1 briefly describes the antitoxic products used. More than 390 guinea-pigs were used in the inoculation tests, carried out by the official methods [1] of standardizing tetanus and diphtheria antitoxins with slight modifications.[2] In table 2 an attempt is made to show the practical relations between toxins and standard antitoxins.

Apparatus.—Only such flasks, delivery pipets and capacity pipets as were tested and approved by the U. S. Bureau of Standards were used. For the detection of slight changes in potency, i.e., a decrease of 10% of the original, 1 c c pipets graduated to 0.01 c c, must be used with an error of less than 0.01 c c. Syringes of the Record type were tested, before use, by weighing the water delivered.

Filtration.—Twenty gm. of diatomaceous earth were mixed with 60 c c of antitoxic product and let stand for varying times. The pasty mixture was then packed around a small Mandler filter, previously sterilized, and filtration was carried out as usual, avoiding evaporation, etc. The numbers of antitoxic units per c c of filtered and original product were compared by the official method of guinea-pig inoculation (see table 4).

Modifications of the Official Technic.—The following departures from the official methods of standardizing tetanus and diphtheria antitoxins were found convenient: (a) Instead of preparing one dose in one syringe for one guinea-pig injection, prepare 2 or 3 times the desired

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[1] Rosenau, M. J.: Hygienic Lab. Bull. 21, 1905. Rosenau, M. J., and Anderson, J. F.: Hygienic Lab. Bull. 43, 1908.

[2] Berg, W. N., and Kelser, R. A.: Jour. Agric. Research, 1918, 13, p. 471.

dose in a sterile, glass stoppered weighing bottle. (b) When injection is made, use a syringe of the Record type, measuring the dose for injection by pushing the plunger from one graduation to another and not from one graduation mark to the end of the barrel. (c) Instead of injecting the dose in a final volume of 4 c c, inject it in a volume of 2 c c.

Chemical Analyses.—Adsorption of protein was looked for in the products injected into guinea-pigs. Determinations were made of (1) total solids, (2) ash, and (3) coagulable protein. The chemical methods have been previously described.[3] The analyses are summarized in tables 3 and 4.

SUMMARY

In every one of 13 filtration experiments with diatomaceous earth, protein was adsorbed from tetanus and diphtheria antitoxic products in quantities ranging from 5 to 33% of the original protein content. In 4 adsorption experiments with fullers' earth, the protein adsorbed was 40 to 78%, depending on conditions.

When the experimental conditions were such that protein adsorption was slight, i.e., 10 to 15%, an adsorption of antitoxic units was not detected.

When protein adsorption was high, i.e., 20 to 78% of the total protein, a corresponding adsorption of 8 to 66% of the original tetanus antitoxic units was detected.

The results indicate that: A rapid laboratory method of testing whether a filter system adsorbs antitoxic units is to estimate, by any convenient chemical method, coagulable protein or solids-not-ash in the original and the filtered product. If there is protein adsorption in large amount, (20% or more), there will be a detectable adsorption of antitoxic units. If protein adsorption is less than 20%, there may be no detectable adsorption of antitoxic units.

A rapid, accurate method of standardizing antitoxins in vitro is much needed, even if only preliminary values are obtained.

It is doubtful whether two workers in separate laboratories would obtain values within 10% of each other in the standardization of the same product by the official method.

As ordinarily carried out, filtration of an immune serum or similar product through a Berkefeld type filter does not result in appreciable losses of antitoxic units.

[3] Berg, W. N.: Jour. Lab. and Clinic Med., 1921, 6, p. 223.

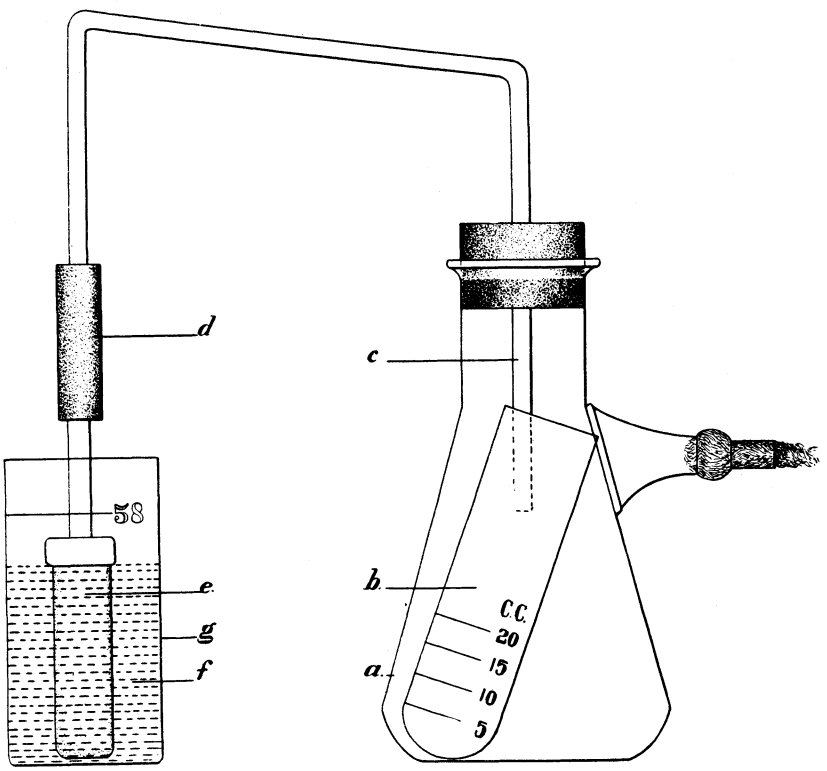

Fig. 1.—a, filter flask capacity 250 c c; b, graduated tube to receive filtrate; c, glass connecting tube; d, heavy rubber tube connection between c and e, small Berkefeld filter; f, pasty mixture of diatomaceous earth, and antitoxic product 60 c c; g, glass weighing bottle, stopper not shown.

TABLE 1

DESCRIPTION OF ANTITOXIC PRODUCTS

Designation of Product	Maker	Potency Stated by Maker in Units per C c	When Obtained	Potency Determined by Writer in Units per C c	Remarks by Maker
Tetanus antitoxin 420 F	Lederle	About 300	December, 1916	300 (1917) 250 (1920)	Berkefeld filtered by maker
Tetanus plasma 9588	Mulford	About 200	March, 1920	500 to 540	Citrated plasma, not filtered, preserved with tricresol
Tetanus serum 2030	Lederle	250 to 300	April, 1920	320 to 340	Serum not filtered, contains no citrate or oxalate, preservative, 0.35% cresol
Standard tetanus antitoxin T 23, T 24	U. S. Hygienic Laboratory	5.0	April, 1920		
Diphtheria antitoxin 615 LN	Lederle	About 800	December, 1916	900 to 1000	Berkefeld filtered
Diphtheria plasma 9004	Mulford	About 400	April, 1920	700 to 750	Citrated plasma, not filtered, preserved with tricresol
Diphtheria serum 2401	Lederle	250 to 300	April, 1920	400 to 450	Not filtered, contains no citrate or oxalate; chloroform 0.4 %
Standard diphtheria antitoxin B 142, B 144, B 145	U. S. Hygienic Laboratory	6.0	March, 1920		

TABLE 2

RELATIONS BETWEEN TOXINS AND STANDARD ANTITOXINS

	Tetanus	Diphtheria
Practical relation between test doses	When each of 4 guinea-pigs is subcutaneously injected with 0.1 unit of antitoxin mixed with 1 test dose of toxin, 3 should die between 72 and 96 hours; 1 close to 96 hours	When each of 4 guinea-pigs is subcutaneously injected with 1 unit of antitoxin mixed with 1 test dose of toxin, 3 should die between 72 and 96 hours; 1 close to 96 hours
Standard antitoxin from Hygienic Laboratory	Glycerolated solution 1 c c contains 5 units	Glycerolated solution 1 c c contains 6 units
Standard weight of guinea-pig....	350 gm.	250 gm.
Test dose of antitoxin injected into 1 guinea-pig	0.1 unit	1 unit
Test Dose (L+ dose) of toxin injected into 1 guinea-pig	0.00075 gm.	0.265 c c
Toxin, from Hygienic Laboratory	Powder	Liquid
Number of minimal lethal doses (MLD) in one test of L+ dose of toxin	100 approximately	201 theoretically,* variable practically

* Hygienic Laboratory Bulletin 21, p. 29.

TABLE 3

ANALYSES OF ANTITOXIC PRODUCTS

Product	Experiment No.	Solids Not Ash in 100 C c		Solids Not Ash Adsorbed, Percentage	Ash in 100 C c		Total Time of Filtration, Hours
		Original, Gm.	Filtered Through Diatomaceous Earth and Berkefeld, Gm.		Original, Gm.	Filtered Through Diatomaceous Earth and Berkefeld, Gm.	
Normal horse serum 2	..	7.86	7.52	4.3	0.780	2.075	..
Tetanus antitoxin 420 F	2	6.74	5.55	17.7	0.68	0.69	2
	3	6.71	4.30	35.9	0.65	1.16	46
Tetanus plasma 9588	4	10.03	9.06	9.7	0.98	1.06	¾
	7	9.90	9.14	8.9	0.93	1.06	24
Tetanus serum 2030	5	8.56	7.85	8.3	0.77	0.70	3½
	6	8.66	7.69	11.2	0.70	0.80	26
Diphtheria serum 2401	21	7.64	7.06	7.6	0.71	0.79	3
	32	....	6.61	13.5	....	0.76	44
Diphtheria plasma 9004	28	9.50	8.74	8.0	0.80	0.95	2½
	31	....	8.64	9.1	....	0.96	48½
Diphtheria antitoxin 615 LN	27	12.27	11.54	5.9	0.91	0.97	6½
	30	....	11.74	4.3	....	1.08	77

TABLE 4

ANALYSES OF ANTITOXIC PRODUCTS

Product	Experiment No.	Coagulable Protein in 100 C c		Coagulable Protein Adsorbed, Percentage	Antitoxic Units Adsorbed, Percentage	Total Time of Filtration, Hours
		Original, Gm.	Filtered Through Diatomaceous Earth and Berkefeld, Gm			
Tetanus antitoxin 420 F	1	6.35	5.73	9.8	..	(few minutes)
	2	....	....	....	8	2*
	3	6.11	4.07	33.3	14	46
Tetanus plasma 9588	4	8.96	8.30	7.4	0	¾
	7	8.96	8.16	8.9	0	24
Tetanus serum 2030	5	7.98	7.22	9.5	0	3½
		8.02	7.42	7.5		
	6	8.02	7.14	11.0	0	26
Diphtheria serum 2401	21	7.14	6.66	6.7	0	3
	32	....	6.30	11.8	0	44
Diphtheria plasma 9004	28	8.86	8.23	7.1	0	2½
	31	....	8.10	8.6	0	48½
Diphtheria antitoxin 615 LN	27	12.12	11.53	4.9	0	6½
	30	.....	11.24	7.3	0	77

* See table 3 for analytic data.